

# PATENT COOPERATION TREATY

TRANSLATION

From the  
INTERNATIONAL SEARCHING AUTHORITY

PCT

WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

To:

Date of mailing **See form PCT/ISA/210**  
(day/month/year)

Applicant's or agent's file reference

**WOB03INRORUS**

**FOR FURTHER ACTION**

See paragraph 2 below

International application No.

**PCT/FR2005/000093**

International filing date (day/month/year)

**14.01.2005**

Priority date (day/month/year)

**15.01.2004**

International Patent Classification (IPC) or both national classification and IPC

**C12N15/53, C12N15/80, C12N9/02, C12N1/15, C12P21/02**

Applicant

**INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE**

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/EP

Authorized officer

Facsimile No.

Telephone No.

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Box No. I

Basis of this opinion

1. With regard to the language, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐

This opinion has been established on the basis of a translation from the original language into the following language

\_\_\_\_\_, which is the language of a translation furnished for the purposes of international search (under Rule 12.3 and 23.1(b)).

2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

a. type of material

☒

a sequence listing

☐

table(s) related to the sequence listing

b. format of material

☒

in written format

☒

in computer readable form

c. time of filing/furnishing

☒

contained in the international application as filed.

☐

filed together with the international application in computer readable form.

☐

furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

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Box No. II

Priority

1. ☐ The following document has not yet been furnished:

☐ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date in the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

**see supplemental sheet**

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Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	6, 7, 10-13	YES
	Claims	1-5, 8, 9, 14-20	NO
Inventive step (IS)	Claims		YES
	Claims	1-20	NO
Industrial applicability (IA)	Claims	1-20	YES
	Claims		NO

2. Citations and explanations:

1. Reference is made to the following documents:

D1: ALVES, ALEXANDRA M. C. R. ET AL, "Highly efficient production of laccase by the basidiomycete *Pycnoporus cinnabarinus*," APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Vol. 70, No. 11, November 2004 (2004-11), pages 6379-6384, XP002341841, ISSN: 0099-2240

D2: MARTINEZ, A.T., "Fungal metalloenzymes oxidizing aromatic compounds of industrial interest," [Online] 25 November 2003 (2003-11-25), XP002295843. Acquired from the Internet.

URL: <<http://www.cib.csic.ex/lignina/pelas/pelas/html>> [acquired 2004-09-10]

D3: LOMASCOLO, A. ET AL, "Overproduction of laccase by a monokaryotic strain of *Pycnoporus cinnabarinus* using ethanol as inducer," JOURNAL OF APPLIED MICROBIOLOGY, Vol. 94, No. 4, 2003, pages 618-624, XP002295716, ISSN: 1364-5072

D4: SIGOILLOT JEAN-CLAUDE ET AL, "Laccase production by a monokaryotic strain of *Pycnoporus cinnabarinus* derived from a

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dikaryotic strain," WORLD JOURNAL OF  
MICROBIOLOGY AND BIOTECHNOLOGY, Vol. 15, No.  
4, August 1999 (1999-08), pages 481-484,  
XP008035159, ISSN: 0959-3993

D5: OTTERBEIN LUDOVIC ET AL, "Molecular cloning  
of the cDNA encoding laccase from *Pycnoporus  
cinnabarinus* I-937 and expression in *Pichia  
pastoris*," EUROPEAN JOURNAL OF BIOCHEMISTRY,  
Vol. 267, No. 6, March 2000 (2000-03), pages  
1619-1625, XP002295717, ISSN: 0014-2956

D6: RECORD ERIC ET AL, "Expression of the  
*Pycnoporus cinnabarinus* laccase gene in  
*Aspergillus niger* and characterization of the  
recombinant enzyme," EUROPEAN JOURNAL OF  
BIOCHEMISTRY, Vol. 269, No. 2, January 2002  
(2002-01), pages 602-609, XP002295718, ISSN:  
0014-2956

2. NOVELTY (PCT Article 33(2))

2.1 The present application fails to comply with the  
requirements of PCT Article 33(1) since the  
subject matter of claims 1-5, 8, 9 and 14-20 does  
not fulfil the criterion of novelty defined in PCT  
Article 33(2).

2.2 Document D3 describes (see entire document) a  
method for preparing endogenous laccase from  
*Pycnoporus cinnabarinus*, the said method being  
performed by overexpression of the encoding gene  
for this protein in a monokaryotic strain of  
filamentous fungi of *P. cinnabarinus* and

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comprises:

- a step for cultivating this monokaryotic strain containing the encoding gene for endogenous laccase, the expression of which is under the control of the endogenous promoter,
- a step for inducing the above-mentioned promoter by adding ethanol,
- the recovery of the laccase.

The expressions "(a given) recombinant (protein)" and "transformed (using an expression vector)" do not make it possible to clearly and unambiguously distinguish the laccase and the strain from the claims of a corresponding laccase and "wild-type" strain.

Expressions such as "if necessary" ["*le cas échéant*"] and "particularly" ["*notamment*"] do not have any limiting effect on the scope of a claim, which amounts to saying that the feature that follows such an expression should be considered entirely optional.

Consequently, the subject matter of claims 1-5, 8 and 18-20 is anticipated by document D3.

Moreover, document D3 explicitly refers to document D5 (see entire document and AF170093 having 100% identity in an overlap of 3331 base pairs with SEQ ID NO: 1 and Q9UVQ2 having 100% identity in an overlap of 518 amino acids with SEQ ID NO: 2), which provides more detailed information on

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certain features; the teaching of this document D5 must therefore be considered to be incorporated in document D3. Consequently, the subject matter of claim 9 is also anticipated by document D3.

2.3 Document D4 describes (see entire document) a method that is similar, but that comprises a step for induction by adding ferulic acid. Thus, the same objections are applicable *mutatis mutandis*.

2.4 Document D5 (see entire document) describes the expression of laccase from *P. cinnabarinus* in a strain of *Pichia pastoris* transformed by means of an expression vector containing the nucleotide sequence represented by SEQ ID NO: 1 that encodes the laccase represented by SEQ ID NO: 2, wherein the expression is placed under the control of the exogenous promoter pAox1.

Since the promoter pAox1 may be considered to be "a promoter corresponding to the sequence SEQ ID NO: 3 or any sequence derived from this promoter by substitution, addition or deletion of one or more nucleotides and retaining the property of being a promoter of the expression of sequences," document D5 describes, in combination, all of the features defined in claims 14-18 and 20. The subject matter of these claims is therefore not novel.

2.5 Document D6 describes (see entire document) the expression of laccase from *P. cinnabarinus* as does D5, but in a strain of *Aspergillus niger* and using

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	<p>the exogenous promoter pgpdA. Thus, the same objections are applicable, <i>mutatis mutandis</i>.</p>
3.	INVENTIVE STEP (PCT Article 33(3))
3.1	<p>The present application fails to comply with the conditions set out in PCT Article 33(1) since the subject matter of claims 1-20 does not involve an inventive step as defined in PCT Article 33(3)).</p>
3.2	<p>Document D3, which is considered to be the closest prior art, describes (see entire document) a method for preparing endogenous laccase from <i>Pycnoporus cinnabarinus</i>, the said method being performed by overexpression of the encoding gene for this protein in a monokaryotic strain of filamentous fungi of <i>P. cinnabarinus</i> and comprises:</p> <ul style="list-style-type: none"><li>- a step for cultivating this monokaryotic strain containing the encoding gene for endogenous laccase, the expression of which is under the control of the endogenous promoter,</li><li>- a step for inducing the above-mentioned promoter by adding ethanol,</li><li>- the recovery of the laccase.</li></ul> <p>The subject matter of the present application is different in that a host cell of <i>P. cinnabarinus</i>, transformed by means of an expression vector, is used for the recombinant preparation of given proteins.</p>
3.3	<p>The problem that the present application proposes</p>



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to solve may be considered to be to provide a method for the recombinant preparation of given proteins. The solution to the problem is the use of a host cell of *P. cinnabarinus* transformed by means of an expression vector.

- 3.4 The solution proposed in the present application (see claims 1-20) is not considered inventive for the following reasons. Document D2 (see paragraph 5) discloses the *P. cinnabarinus* strain as a host cell as well as the expression of endogenous laccase and the expression of an exogenous peroxidase of *Pleurotus eryngii* in this cell. Consequently, in light of the prior art of D3 and D2, it is obvious for a person skilled in the art to use a host cell of transformed *P. cinnabarinus* for the recombinant preparation of given proteins.

The claims do not contain any features which, when combined with the features of any claim to which they refer, satisfy the requirements of the PCT with regard to inventive step.

- 3.5 Moreover, D1 (see boxes II and VI below) is part of the prior art relating to claims 4, 5, 12, 13 and 17, and the claims that refer to these claims, i.e. claims 6, 7 and 18-20. The subject matter of these claims does not involve an inventive step as defined in PCT Article 33(3) since D1 demonstrates (see abstract) that the host cell of *P. cinnabarinus* can successfully be used for the production of other proteins.

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Box No. VI      Certain documents cited

1.    Certain published documents (Rule 43bis.1 and 70.10)

Application No.  
Patent No.

Publication date  
(day/month/year)

Filing date  
(day/month/year)

Priority date (valid claim)  
(day/month/year)

2.    Non-written disclosures (Rule 43bis.1 and 70.9)

Kind of non-written disclosure

Date of non-written disclosure  
(day/month/year)

Date of written disclosure  
referring to non-written disclosure  
(day/month/year)

see form 210

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

**Boxes II and VI**

The priority claimed appears to be inadmissible because the sequences with SEQ ID NOs: 14-18, figures 12 and 13 and claims 4, 5, 12, 13 and 17 are not present in the priority application. Consequently, document D1 is part of the prior art and may be used to evaluate novelty and inventive step in accordance with PCT Article 33(2) and (3).